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Date of Signature and Deposit: 12/8/06

Jean C. Baker, Reg. No. 35,433



**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| Applicants: | Howard J. Jacob, et al. |
| App. No.: | 10/625,870 |
| Filed: | July 23, 2003 |
| Title: | RAT MODEL OF DIABETIC NEPHROPATHY |
| Art Unit: | 1633 |
| Examiner: | Ileana Popa |
| Confirmation No.: | 8005 |
| Docket No.: | 650053.00002 |

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Commissioner for Patents
P O Box 1450
Alexandria, VA 22313-1450

**DECLARATION OF RICHARD J. ROMAN**

Dear Sir:

1. I, Richard Roman, declare that:

2. I am the Director of the Kidney Disease Center and Professor of Physiology and Medicine at the Medical College of Wisconsin, Milwaukee, Wisconsin. I am also a named inventor on the above-identified application.

3. I agree with the Examiner that the marker-assisted breeding scheme disclosed in the specification is complex and requires genotyping and breeding a large number of animals. The results of the breeding program are, however, completely predictable. Our extensive genotyping comparison of theT2DN rat we created (that develops diabetic nephropathy) and the parental GK rat (that is resistant to diabetic nephropathy) indicates that only the mitochondrial genome and 8

genetic markers across 543 tested differ between the strains. Anyone skilled in the art can easily breed a strain of rats with the exact genetic copy of the T2DN rat. Extensive breeding and genotyping is not necessary. All that is required is to follow by PCR the 8 genetic markers that are different between the strains (see specification [0076]) and to backcross the rats with GK rats for several generations until the FHH alleles that are not selected are washed out of the genetic background. This process will require 6-8 generations to complete but no special technology or training is needed to accomplish this goal.

4. In the next paragraphs I summarize a sample breeding scheme that one of skill in the art could employ with information from the specification of the above-identified application. Note that all materials are readily available.

a. Breed a male GK rat with a female FHH rat. The progeny F1 generation will have 1 copy of the FHH gene and 1 copy of the GK alleles at all autosomal loci through the genome and will inherit the mitochondrial genome of the FHH rat from the mother. Both GK rats and FHH rats are inbred strains (genetic twins of the rats we used to create the T2DN). Suitable GK rats are available for purchase from Charles River Laboratories, 251 Ballardvale Street, Wilmington, MA 01887-1000 or Taconic Farms, One Hudson City Centre, Hudson, NY 12534. The FHH strain we used are commercially available from Charles River Laboratories or PhysioGenix Inc., 10437 Innovation Drive, Wauwatosa, WI 53226. (The inventors of the above-identified application are officers and/or employees of PhysioGenix Inc.)

b. Female F1 animals will be mated with a male GK rat to create an N2 generation. The progeny will be genotyped by PCR using the following primers that amplify across simple sequence length polymorphisms in repeated elements in the following 8 genetic markers. D3Rat57, D11Mgh5, D12Rat22, D1Mit18, D1Mit34, D1Mgh12, D1Rat291, and D1Rat185 (see specification [0076]). The exact sequence of each primer and length of the expected product for FHH and GK rats is publicly available on the NIH sponsored Rat Genome Database, RGD@mcw.edu. The primers can be purchased from Invitrogen Corporation.

The rats can be simply genotyped by running the markers on a 4% Metaphor agrose gel and staining the gel with ethidium bromide. 50% of the rats will be heterozygous at any given allele. Five of the markers are on chromosome 1 and are linked. Thus, only 4 markers are independent variables and the odds of obtaining a rat heterozygous at 4 independently inherited markers is 1 in 16. Thus, approximately 60 rats 6-8 breeder pairs will have to be bred in each generation to ensure enough rats are found with the appropriate genotype for breeding the next generation.

c. Female rats that are heterozygous and carry 1 copy of FHH and GK markers at each site will be selected for and bred back to a male GK rat to create the next N3 generation. Again, the pups will be genotyped for the 8 markers to select heterozygotes for the next round of backcross breeding.

d. This process will be repeated for 6 backcross generations. After 6 backcross generation the genetic background will be >98% GK. Male and Female rats heterozygous for FHH alleles at all 8 of the selected genetic markers will be mated. The pups will be genotyped for a male and female that are homozygous for FHH alleles at the 8 selected genetic markers. The line will be propagated by brother/sister mating. At this point the line will remain fixed and nearly genetically identical to the T2DN rats that we created. Given the breeding cycle of rats to reach sexual maturity about 4 litters per year can be generated. Thus, the breeding process will take time approximately 2 years. However, the breeding scheme is very straight-forward and well within the capability of any graduate student in a University genetics lab. Anyone skilled in the art could employ genetic markers in the early generations to speed the process. However, I want to emphasize that this is entirely unnecessary and even simple traditional breeding scheme with minimal genotyping will reproduce the strain with time.

5. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these

statements were made with the knowledge that willful false statements and the like made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Respectfully submitted,

Date: Dr. Richard J. Roman